Synopsis PhD Thesis by Manal Shakeel

Sugar intake elicited locomotor behaviours in flies and honey bees

Introduction

Foraging and food search behaviours are one of the most successful experimental paradigms to study navigation. Insects are highly capable and efficient navigators (Rüdiger 2020; Menzel 2023). Honey bees are unique in that they evolved the capability to communicate the location of profitable food sources to nest mates through dance communication (Frisch 1967). However, the evolution of dance remains largely unresolved (Price and Grüter 2015; Barron and Plath 2017). Interested in the question of how social behaviour or communication evolved from solitary behaviour, Vincent Dethier drew attention to similarities between a local search behaviour in flies and the honey bee dance (Dethier 1957). He was also the first to describe sugar-elicited search behaviour, a particular form of local search that is initiated after taking in a small amount of food. Typical local search behaviour includes a highly tortuous walk with high turning rate, and frequent returns to the location of the sugar drop. This behaviour was studied in other dipterans: *Musca domestica* and *Drosophila melanogaster* (Bell 1984; White et al. 1985; Pereira and Sokolowski 1993). However, the question whether this search behaviour might comprise path integration to return to the location of the food had not been asked. Use of path integration in navigation and homing has been demonstrated in desert ants, dung beetles and bumble bees navigation; and also in mammals. (Muller and Wehner 1988; Chittka et al. 1999; Dacke et al. 2020; Patel et al. 2022; Mittelstaedt and Mittelstaedt 1980).

Additionally, Dethier's idea had not been taken up by bee researchers. This thesis explores the hypothesis that the innate sugar-elicited local search behaviour of solitary insects - which might also be present in social insect foragers - was co-opted during the evolution of social communication of navigational information in honey bee dance. We have established a set up to study sugar-elicited search behaviour in *Drosophila melanogaster* and the western honey bee *Apis mellifera*. To understand the connection between solitary sugar-elicited behaviour and social dance communication, I studied initiation and regulation of local search in both the species, and compared that to honey bee dance. Furthermore, I tested whether local search involves path integration to guide returns to the location of food reward. The role of self-

motion cues in the local search was also explored to understand the mechanistic basis of local search.

Aims and objectives:

1. To understand the connection between solitary sugar-elicited behaviour and social dance communication

   I wanted to explore Dethier's idea that honey bee dance evolved from the walking pattern of local search in solitary flies. Using a comparative approach, local search in flies and honey bees after sugar intake was studied. I then explored the temporal dynamics of initiation of local search and dance in flies and honey bees, respectively.

   Following questions were investigated:

   a. Do *Drosophila melanogaster* males and *Apis mellifera* foragers initiate local search after sugar intake?

   b. What is the importance of the location of food reward in the behaviour?

   c. How long does the motivational effect of the food reward last after sugar intake for initiating local search?

   d. What are the temporal dynamics of initiating dance after sugar collection in honey bee foragers

2. To study the mechanistic basis of sugar-elicited search using *Drosophila*

   Interested in the navigational aspect of the behaviour, the role of path integration and proprioceptive inputs from the leg was explored.

   This aim had the following objectives:

   a. To identify the navigational strategy that flies use to return to the location of the sugar: external cues vs path integration

   b. To investigate the role of mechanosensory inputs from legs in path integration-based local search

Description of the work

To address aim 1: Behavioural response of male hungry flies and honey bee nectar foragers to sugar stimulus was studied in a lab setup to study local search. The trajectories were video recorded and analysed. As a control, no sugar stimulus was provided to test animals and their response was recorded. Sugar elicited local search in both the species, and the search behaviour involved path integration and increased turning. Using a passive displacement experiment- by feeding the fly/bee in one location and transferring them to a new arena before they started walking, I tested whether the location of the stimulus could be separated from the search behaviour. Upon transfer, flies and bees started a search around the location where they were released. The origin of the search was the location where they start walking, and is independent from the sugar location. A series of delay experiments was then used to study the temporal dynamics of initiation of search involving path integration in flies and bees by impeding walking after sugar intake for different time durations. The motivation to initiate search that involves path integration lasted for 3 min in both the species. A similar delay experiment in honey bee foragers after sugar collection revealed that the modulatory effect of sugar was sustained for 15 min. Initiation of both these behaviours showed a linear decline when immobilized after sugar intake. This work will constitute chapters 2 and 3 of the thesis.

To address aim 2: To distinguish whether flies use external sensory cues or self-motion cues to return to the location of the sugar drop, the behaviour was recorded in the absence of visual and chemosensory cues. In the absence of any external cues, flies use path integration to guide their way to the location of food. Passive displacement experiments confirmed this result. This work will constitute a portion of chapter 2 of the thesis. Mechanosensory receptor (Nanchung) compromised flies were used to explore how mechanosensory inputs would feed into path integration while performing local search. Mutant flies showed a defect in returning to the origin. A manipulation experiment in which the legs of the flies were clipped and then recorded after sugar intake was done to study the role of impaired feedback from legs. Search trajectories of leg clipped flies did not show an effect on the returns but showed two phenotypes: small clusters of searches in addition to the origin and scale-free walking pattern (Lévy walk). These experiments will form chapter 4 of the thesis.

Conclusions and future directions:

Local search relies on path integration to return to the location of the food (Kim and Dickinson, 2017; Brockmann et al., 2018). Flies and honey bee foragers initiated a sugar-elicited local search involving path integration and increased turning (Brockmann et al., 2018). Our collaborators have demonstrated that this path integration-controlled search behaviour is initiated by pharyngeal and not peripheral taste receptor cells (Murata et al., 2017). Using a comparative approach, I then explored the temporal dynamics of initiation of local search and dance in flies and honey bees, respectively (Shakeel and Brockmann, 2023). Passive displacement experiments showed that stimulus (sugar) and behavioural response (local search) can be spatially and temporally dissociated in both species. Sugar intake increases the probability of initiating a path integration-based search, but the search begins at the onset of walking. Our findings suggest that the search itself is composed of two independent behavioural responses: a turning behaviour that helps to explore the area around the food location and a temporally limited path integration guided search that keeps the animal in the proximity of the food item found. I also demonstrated that this heightened motivation to initiate such a search lasted for 3 min in both the species by introducing delay between feeding and behavioural response. A similar delay experiment with foraging honey bees led to a significant reduction in the probability to initiate dance behaviour after 15 min while the number of circuits significantly declined at 3 min. This work also explored how the inputs from legs affect walking and path integration during local search. Nanchung mutants showed defects in returns, while legs clipped flies showed additional clusters of search and Lévy walks.

We propose that the food-intake during foraging has the capability to activate the path integration system in flies and honey bees, and this interaction might have been elaborated during evolution to guide the walking pattern of the honey bee dance. Additionally, this study supports the idea that we can use local search as a paradigm to study initiation and control of path integration system using flies. Since neurogenetic manipulations are difficult in honey bees, one can use *Drosophila* to gain insights into the neural pathways involved in dance communication in honey bees.

References:

Barron AB and Plath JA (2017). The evolution of honey bee dance communication: A mechanistic perspective. Journal of Experimental Biology, 220(23), 4339–4346.

Bell WJ, Tortorici C, Roggero R, Kipp L and Tobin TR (1985). Sucrose-stimulated search orientation in *Drosophila melanogaster*: modulation by period of deprivation. Animal Behaviour, 33, 436–448.

Brockmann A, Basu P, Shakeel M, Murata S, Murashima N, Boyapati RK, Prabhu NG, Herman JJ and Tanimura T (2018). Sugar intake elicits intelligent searching behavior in flies and honey bees. Frontiers in Behavioral Neuroscience, 12, 280.

Chittka L, Williams N, Rasmussen H and Thomson J (1999). Navigation without vision: bumblebee orientation in complete darkness. Proceeding of Royal Society B, 266, 45–50.

Dacke M, el Jundi B, Gagnon Y, Yilmaz A, Byrne M and Baird E (2020). A dung beetle that path integrates without the use of landmarks. Animal Cognition. 23, 1161-117

Dethier VG (1957). Communication by insects: Physiology of dancing. Science 125, 331–336.

Frisch KV (1967) The dance language and orientation of bees. Harvard University Press, Cambridge.

Hansson L-A (ed.) and Akesson S (ed.) (2014) Animal Movement Across Scales. Oxford: Oxford University Press.

Kim, IS and Dickinson, MH (2017). Idiothetic Path Integration in the Fruit Fly Drosophila melanogaster. Current Biology, 1–12.

Menzel R (2023) Navigation and dance communication in honeybees: a cognitive perspective. Journal of Comparative Physiology A

Mittelstaedt ML and Mittelstaedt H (1980). Homing by path integration in a mammal. Naturwissenschaften. 67, 566-567.

Murata S, Brockmann A, and Tanimura T (2017). Pharyngeal stimulation with sugar triggers local searching behavior in *Drosophila*. J. Exp. Biol. 220, 3231-3237.

Patel RN, Kempenaers J and Heinze S (2022). Vector navigation in walking bumblebees. Current Biology. 32, 2871-2

Pereira HS and Sokolowski MB (1993). Mutations in the larval foraging gene affect adult locomotory behavior after feeding in Drosophila melanogaster. Proceedings of the National Academy of Sciences of the USA, 90(11), 5044–5046.

Price RIA and Grüter C (2015). Why, when and where did honey bee dance communication evolve? Frontiers in Ecology and Evolution, 3(Nov), 1–7.

Shakeel M and Brockmann A (2023). Temporal effects of sugar intake on fly local search and honey bee dance behaviour, bioRxiv 2023.03.13.532281

Wehner R (2020) Desert Navigator. The Journey of an Ant. Harvard University Press.

White J, Tobin TR and Bell WJ (1984). Local search in the housefly *Musca domestica* after feeding on sucrose. Journal of Insect Physiology, 30(6), 477–487.